UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

DATE: May 17, 2011

SUBJECT: Pyroxasulfone: Second Report of the Cancer Assessment Review Committee

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FROM: Jessica Kidwell, Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509P)

THROUGH: Jess Rowland, Chair
Cancer Assessment Review Committee
Health Effects Division (7509P)

TO: Abdallah Khasawinah, Toxicologist
RAB IV, Health Effects Division (7509P)

Michael Walsh, RM 23
Kathryn Montague
Herbicide Branch, Registration Division (7505P)

The Cancer Assessment Review Committee met on April 13, 2011 to re-evaluate the cancer classification of Pyroxasulfone in accordance with the *EPA's Final Guidelines for Carcinogen Risk Assessment* (March, 2005). Attached please find the final Cancer Assessment Document.

CANCER ASSESSMENT DOCUMENT

SECOND EVALUATION OF THE CARCINOGENIC POTENTIAL OF **PYROXASULFONE**

PC CODE 090099

May 17, 2011

CANCER **A**SSESSMENT **R**EVIEW **C**OMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

PYROXASULFONE CANCER ASSESSMENT DOCUMENT FINAL

DATA PRESENTATION: _____
Abdallah Khasawinah, Toxicologist

DOCUMENT PREPARATION: _____
Jessica Kidwell, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise noted.)

Gregory Akerman _____

Marion Copley _____ for (MC)

Kit Farwell _____

Ray Kent _____

Mary Manibusan _____

Nancy McCarroll _____

Karlyn Middleton _____

Jess Rowland, Chair _____

P.V. Shah _____

NON-COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the pathology report)

John Pletcher, Consulting Pathologist _____

OTHER ATTENDEES: Michael Walsh (RD), Kathryn Montague (RD), Jessica Ryman (HED/RAB IV), Becky Daiss (HED/RAB IV); On conference call with Global Review Partner-Canada PMRA (Catherine Adcock, Carmen Cheung, Charles Smith)

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EXECUTIVE SUMMARY

On April 13, 2011, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs met to re-evaluate the carcinogenic potential of pyroxasulfone. This is the second time this chemical has been evaluated by the CARC. The toxicological database was jointly reviewed by Australian (APVMA), Canadian (PMRA), and United States (EPA) regulatory agencies.

In 2010, the CARC classified pyroxasulfone as a "Likely to be Carcinogenic to Humans". The weight-of-evidence for this classification included the occurrence of rare transitional cell papilloma and carcinoma of the urinary bladder in male rats, and rare renal tubular adenomas in male mice. At that time the Committee recommended that a linear low-dose approach (Q_1*) for assessing cancer risk in humans should be based on combined urinary bladder tumors in male rats. This extrapolation, rather than an RfD approach, was warranted due to lack of data on mode of action (MOA). Subsequently, the Registrant submitted additional studies to support a mode of action for bladder tumors and a re-evaluation of the kidney tumors and requested that the Agency re-assess the cancer classification of pyroxasulfone based on this new information.

At the re-evaluation, the Committee considered the following for a weight-of-evidence determination on the carcinogenic potential of pyroxasulfone:

Carcinogenicity

Rats

- *Urinary Bladder Tumors:* Administration of pyroxasulfone resulted in transitional cell bladder tumors in male rats. Dr. Samuel Cohen, an international expert in the field of renal pathology, re-evaluated the histopathology findings from the chronic toxicity/carcinogenicity rat study and confirmed the tumor findings, except for one carcinoma at the 1000 ppm dose that he diagnosed to be an adenoma. Male rats had statistically significant trends for urinary bladder transitional cell papillomas and combined papillomas and carcinomas, both at $p < 0.01$. Although not significant by pair-wise comparison at the top two doses, the incidence of urinary (urothelial) papillomas in male Crl:CD (CD) rats at 1000 ppm (6%) and 2000 ppm (8%) exceeded the historical control range of 0-3.33% from the animal provider (Charles River Laboratories) and the laboratory historical control. The urinary bladder transitional cell tumors are considered to be rare tumors. Supporting evidence for these tumors included preneoplastic lesions (mucosal hyperplasia and inflammation) seen at 1000 and 2000 ppm. **The CARC considered the transitional cell bladder tumors in male rats to be treatment-related.**

Mouse

- *Kidney Tumors:* Kidney tubule adenomas occurred in 1/49 and 3/44 male mice administered 5 and 2000 ppm of pyroxasulfone in the diet. None were reported at the mid-dose of 150 ppm or the controls. Dr. Gordon Hard, an international expert in the field of mouse kidney tumors, re-evaluated the histopathology findings from the chronic mouse study along with 14- and 90-day mouse studies and confirmed the tumor findings. However, Dr Hard concluded that these tumors were spontaneous and not treatment-related. A Pathology Working Group (PWG) of five prominent pathologists including the study pathologist and Dr. Hard and chaired by Dr. Hardisty reaffirmed Dr. Hard's findings and conclusions. These conclusions were based on the following considerations: 1) Absence of any cytotoxicity (degeneration or individual cell necrosis) in studies ranging from 14 days to 18 months at doses up to 15,000 ppm; 2) Absence of cell regeneration leading to precursor lesions such as atypical tubular hyperplasia at all time points and doses up to 15,000 ppm; 3) Lack of exacerbation of chronic progressive nephropathy, a spontaneous disease in rodents that results in cell regeneration which can result in renal tubule tumors in chronic studies; and 4) Lack of a clear dose response in the distribution of tumors between test substance treated groups. **The CARC considered the evidence presented in this PWG re-evaluation and concurred with the PWG conclusion that the kidney adenomas in male mice were not treatment-related.**

Mutagenicity

- There is no concern for mutagenicity.

Mode of Action (Male Rat Urinary Bladder Tumors)

- The postulated Mode of Action (MOA) for urinary bladder tumors in male rats is a non-genotoxic process producing increased cell proliferation resulting from site-specific cytotoxicity and the associated compensatory regenerative response leading to hyperplasia and subsequent tumors in the urinary bladder. The series of events leading to urinary bladder tumors (transitional cell papillomas) are initiated by the formation of crystals in urine and the formation of calculi which induce a hyperplastic (preneoplastic) response in the urinary bladder epithelium. Urinary bladder epithelial hyperplasia is a regenerative response resulting from irritation and inflammation caused by an abrasive action of calculi on the urinary bladder epithelium. Urinary bladder lesions that precede or accompany epithelial hyperplasia may include inflammation (acute or chronic), ulceration, and necrosis. Crystal formation in the absence of calculi is not associated with hyperplasia or urinary bladder tumors; therefore, the formation of urinary bladder calculi is the prerequisite for subsequent hyperplasia and neoplasia. The requirement for calculi formation also supports high-dose threshold phenomenon for the development of urinary bladder tumors, i.e., tumors do not develop at doses too low to produce calculi. The CARC concluded that the mode of action for bladder tumors has been adequately established. The data presented supported both a dose-response and temporal

concordance of the key events and bladder tumors.

Classification and Quantification of Carcinogenic Potential

In accordance with EPA's *Final Guidelines for Carcinogen Risk Assessment (March 2005)*, the CARC classified Pyroxasulfone as "Not Likely to be Carcinogenic to Humans" at doses below those that cause urinary bladder calculi formation resulting in cellular damage of the urinary tract. Crystal formation in the absence of calculi is not associated with hyperplasia or urinary bladder tumors; therefore, the formation of urinary bladder calculi is the prerequisite for subsequent hyperplasia and neoplasia. The requirement for calculi formation also supports a high-dose threshold phenomenon for the development of urinary bladder tumors, i.e., tumors do not develop at doses too low to produce calculi. No treatment-related tumors were seen in mice. There is no concern for mutagenicity.

The Agency has determined that the quantification of risk using a non-linear approach (i.e., RfD) will adequately account for all chronic toxicity, including carcinogenicity, that could result from exposure to pyroxasulfone. There is a clear threshold of 1000 ppm (42.55 mg/kg/day) for tumorigenesis. A point of departure (POD) of 50 ppm (2.05 mg/kg/day) is not expected to result in urinary bladder calculi formation which is a prerequisite for subsequent hyperplasia and neoplasia.

I. BACKGROUND INFORMATION

On March 31, 2010, the Cancer Assessment Review Committee of the Health Effects Division of the Office of Pesticide Programs met for the first time to evaluate the carcinogenic potential of pyroxasulfone. The toxicological database was jointly reviewed by Australian (APVMA), Canadian (PMRA), and United States (EPA) regulatory agencies.

At this 2010 meeting Abdallah Khasawinah of Risk Assessment Branch IV presented the chronic toxicity/carcinogenicity study in Crl:CD®(SD)IGS BR rats and the carcinogenicity study in Crl:CD1(ICR) mice. Pyroxasulfone (99.1% a.i.) was administered in the diet to 70 young (six to eight-week-old) rats/sex/group at dose levels of 0, 5, 50, 1000, or 2000 ppm (equivalent to 0/0, 0.21/0.28, 2.05/2.69, 42.55/54.28, or 84.58/106.74 mg/kg bw/day in males/females) for at least 23 months. Surviving animals were sacrificed at 99 weeks (males), or 97 weeks (females). Pyroxasulfone (99.1% w/w a.i.) was administered in the diet to mice (60/dose) at dietary doses of 0, 5, 150, 2000 ppm or 4500 ppm (males)/4000 ppm (females) (equivalent to 0, 0.62/0.84, 18.4/25.5, 228/306, and 465/668 mg/kg/day in males/females) of the test substance for up to 18 months. Treatment at 4500/4000 ppm exceeded the maximum tolerated dose, and this group was terminated prior to the scheduled sacrifice. After approximately 14 months on study, dietary concentrations for the 2000 ppm groups were reduced to 1000 and 500 ppm for males and females, respectively, in order to insure a satisfactory survival rate at the scheduled sacrifice. To provide interim data at one year, 10 mice per sex per group were sacrificed and given gross and microscopic pathological examinations. After approximately 18 months of dietary exposure, all surviving mice were sacrificed and given a gross and microscopic pathological examination. Information on mutagenicity, structure activity relationship was also presented.

At the March 31, 2010 meeting, the CARC concluded the following:

"Carcinogenicity

Rat

• Administration of pyroxasulfone resulted in transitional cell bladder tumors in male rats. Male rats had statistically significant trends for urinary bladder transitional cell papillomas and combined papillomas and carcinomas, at $p < 0.01$. Although not significant by pair-wise comparison at the top two doses, the incidence of urinary (urothelial) papillomas in male Crl:CD (CD) rats at 1000 ppm (4%) and 2000 ppm (8%) exceeded the historical control range of 0-3.33% from the animal provider (Charles River Laboratories). The urinary bladder transitional cell tumors are considered to be rare tumors. Supporting evidence for these tumors include preneoplastic lesions (mucosal hyperplasia and inflammation) seen at 1000 and 2000 ppm. **The CARC considered the transitional cell bladder tumors in male rats to be treatment-related.**

• The **thyroid follicular cell tumors seen in male rats were <u>not</u> considered to be treatment-related.** While there were significant trends for adenomas and combined tumors, the incidence

of adenomas (6%) and carcinomas (2%) at the high dose were within the historical control range of 1.67-12% for adenomas and 0.87-3.85% for carcinomas from the animal provider (Charles River Laboratories).

• **The adrenal gland tumors (adenomas only) seen in female rats were not considered to be treatment-related.** While there was a significant trend for adrenal gland cortical adenomas and a significant pair-wise comparison of the 2000 ppm dose group with the controls, the incidence at the high dose (5%), was within the historical control range of 1.43-12% from the animal provider (Charles River Laboratories).

• **The mammary gland tumors seen in female rats were not considered to be treatment-related.** While there were significant pair-wise comparisons of the 1000 ppm dose group with the controls for mammary gland adenomas and combined adenomas and carcinomas and a significant pair-wise comparison of the 5 ppm dose group with the controls for mammary gland combined adenomas and carcinomas at $p < 0.05$, there was no dose response for these tumors. The incidence of both adenomas (25% and 16%) and carcinomas (24% and 20%) at the 1000 ppm and 2000 ppm dose groups, respectively, were within the historical control range of the animal provider (1-32% adenomas; 9-58% carcinomas).

• Adequacy of Dosing: Dosing at the high dose was considered adequate, but not excessive, on the basis of reduced body weight and body weight gain and histopathological findings in the heart, urinary bladder, sciatic nerve, and liver.

Mouse

• Administration of pyroxasulfone resulted in kidney tumors (adenomas only) in male mice. Male mice had a statistically significant trend for renal tubular adenomas at $p < 0.05$. The incidence of adenomas at the high dose (7%) exceeded the historical control range of 2-4% from the animal provider (Charles River Laboratories). This tumor is supported by non-neoplastic lesions of the kidney, including intratubular precipitate and tubular hyperplasia. This tumor is considered to be rare. **The CARC considered the kidney tumors to be treatment-related.**

• Adequacy of Dosing: Dosing at the high dose of 2000 ppm was considered to be adequate, but not excessive, in both sexes of mice based on an increase in the incidence of histopathology of the kidney and nervous tissue.

Mutagenicity: There is no concern for mutagenicity.

Structure-Activity Relationship: There are no adequate analogues to support SAR for pyroxasulfone.

Mode of Action: No satisfactory mode of action data were submitted for the March 31, 2010 meeting.

Classification and Quantification of Carcinogenic Potential at the March 31, 2010 CARC meeting

In accordance with the EPA's *Final Guidelines for Carcinogen Risk Assessment* (March, 2005), the CARC classified pyroxasulfone as **"Likely to be Carcinogenic to Humans"** based on rare tumors in two species: a treatment-related increase in urinary bladder tumors in male rats and kidney tumors in male mice, and with incidences exceeding that of the animal supplier's historical controls. The non-neoplastic lesions in the rat bladder and mouse kidney support the findings that the tumors are treatment-related. There is no mutagenic concern for pyroxasulfone.

The Committee recommended a linear low-dose approach (Q_1*) for assessing human cancer risk based on combined urinary bladder tumors in male rats."

For complete details of the March 31, 2010 meeting refer to HED TXR# 0055356.

II. INTRODUCTION

The "Likely to be Carcinogenic to Humans" classification of pyroxasulfone by the CARC at its March 31, 2010 meeting prompted the registrant Kumiai Chemical Industry C., Ltd. (Kumiai), represented by Landis International Inc., to submit an MOA for bladder tumors in male rats and an expert re-evaluation of the kidney tumors in male mice. They submitted a request for the HED CARC to consider the new data for potential re-classification of pyroxasulfone carcinogenicity. Kumiai submitted the additional following documents:

1. Cohen, S.; Gale, E.; Abe, K.; et al. (2010) Pyroxasulfone Mode of Action: Weight-of-Evidence for Carcinogenicity: (Mice). Unpublished study prepared by Kumiai Chemical Industry Co., Ltd. 41 p., MRID: 48354401.

2. Hardisty, J. (2011) Pathology Working Group to Examine Histopathologic Changes Reported in the Kidneys of Mice in Toxicology and Carcinogenicity Studies with Pyroxasulfone (KIH-485 TGAI): Final Report. Project Number: 912/001, 16203, B693/001/540. Unpublished study prepared by Experimental Pathology Labs., Inc. 245 p., MRID: 48354405.

3. GORDON C. HARD. 2010. Expert Report on Kidney Histopathology in Toxicology/ Carcinogenicity Studies with KIH-485 TGAI (Pyroxasulfone) Administered in the Feed to CD-1 Mice. September 29, 2010. Sponsor: Kumiai Chemical Industry Co., ltd., c/o Landis International, Inc., MRID 48354403.

4. Cohen, S.M. 2011. Review of selected slides from the bladders of male rats from the two-year carcinogenicity bioassay on pyroxasulfone. Report no. 2011-01. Sponsor: Kumiai Chemical Industry Co., ltd., c/o Landis International, Inc., MRID 48354406.

5. Tsuboi, M. (2011) Electron Microscopic Examination of Rat Urinary Bladder Treated with Pyroxasulfone (KIH-485) for 14 Days: Revised Report. Project Number: C014/001/567. Unpublished study prepared by Biosafety Research Center. MRID: 48354402. 63 p.

6. Cohen, S.; Arnold, L. The Effect of Dietary Administration of Pyroxasulfone on the Urinary Bladder of Male Rats: Final Report. Project Number: 267C/125, 310. Unpublished study prepared by University of Nebraska. 114 p., MRID: 48354404.

The second CARC meeting on April 13, 2011 focused on the tumors of concern namely: urinary bladder tumors in male rats, with an accompanying MOA for bladder tumors, and renal tubular adenomas in male mice.

III. RE-EVALUATION OF CARCINOGENICITY STUDIES

1. Combined Chronic Toxicity/Carcinogenicity Study in Rats

Reference: Munley, S. M. (2009) KIH-485TGAI: Two-Year Carcinogenicity Feeding Study in Rats. DuPont Haskell Global Centers for Health and Environmental Sciences, E.I. DuPont de Nemours & Company, Delaware, USA. DuPont-18416. February 9, 2009. Unpublished. MRID # 47701709.

A. Experimental Design

Pyroxasulfone (99.1% a.i.) was administered in the diet to 70 young (six to eight-week-old) Crl:CD®(SD)IGS BR rats/sex/group at dose levels of 0, 5, 50, 1000, or 2000 ppm (equivalent to 0/0, 0.21/0.28, 2.05/2.69, 42.55/54.28, or 84.58/106.74 mg/kg bw/day in males/females) for at least 23 months. Surviving animals were sacrificed at 99 weeks (males), or 97 weeks (females).

B. Discussion of Tumor Data

Urinary Bladder Papillomas:

The study pathologist diagnosed 1, 0, 0, 3 and 5 papillomas in the controls, 5 ppm, 50 ppm, 1000 ppm and 2000 ppm treatments, respectively and one urinary bladder transitional cell carcinoma in one male rat administered 1000 ppm of pyroxasulfone (Table 1). A re-evaluation by an independent expert pathologist confirmed the findings of the study pathologist except for the carcinoma which was considered to be an adenoma (MRID 48354406) (Table 2). The male rat bearing this tumor was found dead on day 469 with severe urinary calculus/calculi. The expert pathologist stated "The diagnosis of carcinoma was made probably because of the apparent invasion of the lesion into the muscle wall of the bladder. However, the epithelium is clearly benign in appearance throughout this bladder. There is extensive calculus material in the lumen which is also present in the lumen of the lesion extending through the wall of the bladder. The staining properties are indicative of calcium-containing material in these calculi and crystals. Furthermore, this animal had marked hydronephrosis of the kidney. Thus, it is apparent that this animal had obstructive urinary tract disease secondary to these calculi, resulting in hydronephrosis of the kidneys and also resulting in a diverticulum of the urinary bladder. The extension through the muscle wall actually represents a diverticulum rather than invasion by malignant tumor. Secondary to the presence of the calcified material there is extensive proliferation of the bladder epithelium, both within the lumen and in the portion extending into the wall. In one portion of the slide, one can actually see the extension from the surface into the bladder wall of this proliferative epithelium." The CARC's consulting pathologist Dr. John Pletcher reviewed the independent expert pathologist's report and concurred with the findings (TXR No. 0055884).

Table 1. Pyroxasulfone – Crl:CD(SD)IGS BR Rat Study (MRID 47701709)
Male Urinary Bladder Transitional Cell Tumor Rates[+] and Fisher's Exact Test and Exact Test for Trend Results **(Original Study Pathologist Diagnosis)**

	Dose (ppm)				
	0	5	50	1000	2000
Papillomas (%)	1/67 (1)	0/65 (0)	0/70 (0)	3/68 (4)	5[a]/66 (8)
p =	0.0023**	1.0000	1.0000	0.3153	0.1006
Carcinomas (%)	0/67 (0)	0/65 (0)	0/70 (0)	1[b]/68 (1)	0/66 (0)
p =	0.3988	1.0000	1.0000	0.5037	1.0000
Combined (%)	1/67 (1)	0/65 (0)	0/70 (0)	4/68 (6)	5/66 (8)
p =	0.0022**	1.0000	1.0000	0.1875	0.1006

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52.
[a]First papilloma observed at week 58, dose 2000 ppm.
[b]First carcinoma observed at week 67, dose 1000 ppm.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 2. Pyroxasulfone – Crl:CD(SD)IGS BR Rat Study (MRID 47701709)
Male Urinary Bladder Transitional Cell Tumor Rates[+] and Fisher's Exact Test and Exact Test for Trend Results
(Re-Evaluation Diagnosis by Expert Pathologist MRID 48354406)

	Dose (ppm)				
	0	5	50	1000	2000
Papillomas (%)	1/67 (1)	0/65 (0)	0/70 (0)	4/68 (6)	5[a]/66 (8)
Carcinomas (%)	0/67 (0)	0/65 (0)	0/70 (0)	0/68 (0)	0/66 (0)
Combined (%)	1/67 (1)	0/65 (0)	0/70 (0)	4/68 (6)	5/66 (8)
p =	0.0022**	1.0000	1.0000	0.1875	0.1006

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52.
[a]First papilloma observed at week 58, dose 2000 ppm.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

The incidence of urinary (urothelial) papilloma in male Crl:CD (CD) rats was reported by the animal provider (Charles River Laboratories) to be 1.43-3.33%. The incidence of urinary (urothelial) carcinoma in male Crl:CD (CD) rats was reported by the animal provider (Charles River Laboratories) to be 1.67-2.00%.
http://www.criver.com/SiteCollectionDocuments/rm_rm_r_lesions_survival_crlcd_sd_rats.pdf

The incidence of urinary (urothelial) papilloma in male Crl:CD (CD) rats by the conducting laboratory (DuPont Haskell Labs) to be 0-3.33%. The incidence of urinary (urothelial) carcinoma in male Crl:CD (CD) rats by the conducting laboratory (DuPont Haskell Labs) to be 0-1.67%. (MRID 48430004)

C. Non-Neoplastic Histopathological Findings in the Urinary Bladder

Urinary Bladder

In males, increased ($p<0.01$) incidences of minimal to severe mucosal hyperplasia were noted in the 1000 ppm (31%) and 2000 ppm (61%) groups compared to the controls (6%), and an increased ($p<0.01$) incidence of minimal to mild mucosal inflammation was observed at 2000 ppm (14%) compared to the controls (4%).

Mucosal Hyperplasia

In males, mucosal hyperplasia of the urinary bladder, including both focal/multifocal and diffuse

lesions, was observed in 4/70, 5/69, 2/70, 22/70, and 43/70 rats fed diets containing 0, 5, 50, 1000, and 2000 ppm of the test substance, respectively. Both the incidence and severity of mucosal hyperplasia were increased in males at 1000 ppm and above.

Mucosal hyperplasia of the urinary bladder was characterized by a focal, multifocal, or diffuse increase in the thickness of the transitional epithelium (urothelium) lining the urinary bladder lumen. The hyperplasia was identical to that observed in rats secondary to either urinary tract infection-associated inflammation or bladder calculi-associated irritation. These latter two conditions (urinary tract infections and calculi) account for the background incidence of bladder mucosal hyperplasia.

In females, the incidence and severity of urinary bladder mucosal hyperplasia was similar across all dose groups, including the controls. Therefore, mucosal hyperplasia was not test substance related in this sex.

Mucosal Inflammation

In males, mucosal inflammation of the urinary bladder was observed in 3/70, 2/69, 0/70, 6/70, and 10/70 rats fed diets containing 0, 5, 50, 1000, and 2000 ppm of the test substance, respectively. Both the incidence and severity of mucosal inflammation were increased in males at 1000 ppm and above.

The urinary bladder mucosal inflammation was characterized by the presence of mixed inflammatory cells in the transitional epithelium and lamina propria of the bladder mucosa. In most cases, the mucosal inflammation was seen in association with mucosal hyperplasia; however, the relationship of the two lesions was not clear.

In females, urinary bladder mucosal inflammation was only present in four rats, each in a different dose group. Therefore, mucosal inflammation was not test substance related in females.

2. Carcinogenicity Study in Mice

Reference: Munley, S. M. 2009. KIH-485TGAI: Oncogenicity Eighteen-Month Feeding Study in Mice. DuPont Haskell Global Centers for Health and Environmental Sciences, E.I. DuPont de Nemours & Company, Delaware, USA. DuPont Report No.:18417. January 26, 2009 Unpublished MRID: 47701710

A. Experimental Design

Pyroxasulfone (99.1% w/w a.i.) was administered in the diet to Crl:CD1(ICR) mice (60/dose) at dietary doses of 0, 5, 150, 2000 ppm or 4500 ppm (males)/4000 ppm (females) (equivalent to 0, 0.62/0.84, 18.4/25.5, 228/306, and 465/668 mg/kg/day in males/females) of the test substance for up to 18 months. Treatment at 4500/4000 ppm exceeded the maximum tolerated dose, and this

group was terminated prior to the scheduled sacrifice. After approximately 14 months on study, dietary concentrations for the 2000 ppm groups were reduced to 1000 and 500 ppm for males and females, respectively, in order to insure a satisfactory survival rate at the scheduled sacrifice. To provide interim data at one year, 10 mice per sex per group were sacrificed and given gross and microscopic pathological examinations. After approximately 18 months of dietary exposure, all surviving mice were sacrificed and given a gross and microscopic pathological examination. Assessment of toxicity was based on mortality, clinical observations, and clinical and anatomic pathology evaluations.

B. Discussion of Tumor Data

Tumor Analyses: Mice

Incidence of renal tubular adenomas as diagnosed by the study pathologist and reported in the study is listed in Table 3. According to this diagnosis, male mice had a statistically significant trend for renal tubular adenomas at $p < 0.05$. There were no significant pair-wise comparisons of the dosed groups with the controls. The statistical analyses of the male mouse tumors were based upon Fisher's Exact Test for pair-wise comparisons the Exact Test for trend since there were no statistically significant trends for mortality.

Table 3. Pyroxasulfone – Crl:CD-1(ICR) Mouse Study (MRID 47701710) Male Renal Tubular Tumor Rates[+] and Fisher's Exact Test and Exact Test for Trend Results (Study Pathologist Diagnosis)

	Dose (ppm)			
	0	5	150	2000
Adenomas# (%)	0/44 (0)	1[a]/49 (2)	0/47 (0)	3/44 (7)
p =	0.03045*	0.52688	1.00000	0.12069

+Number of tumor bearing animals/Number of animals examined, excluding interim sacrifice animals and those animals that died before week 54.
#No renal tubular carcinomas observed.
[a]First adenoma observed at week 78, dose 5 ppm.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Laboratory historical control data from 14 studies by the conducting laboratory for this type of tumor was 0-1% (MRID 48430005). One renal adenoma was identified in the 14 studies. The incidence of kidney adenoma/tubular adenoma in male CD1 (ICR) mice was reported by the animal provider (Charles River Laboratories) to be 0-4%., See:
http://www.criver.com/SiteCollectionDocuments/rm_rm_r_lesions_crlcd_1_icr_mouse.pdf

Tumor Revaluation:

Revaluation of the renal tubular adenomas of the male mice was conducted by an independent kidney expert pathologist and reviewed by a Pathology Working Group (MRID 48354405).

The reviewing pathologist (Hard, 2010: MRID 48354403) examined the 18-month mouse oncogenicity study along with a 14-and 90-day mouse studies and concluded that the renal tubular adenomas observed in male mice were of spontaneous origin. His findings showed that the administration of pyroxasulfone was associated at the higher exposure levels with an ascending form of nephropathy diagnosed as retrograde nephropathy (RGN) presumably arising secondarily from an effect in the lower urinary tract, possibly the formation of urinary solids.

Pyroxasulfone did not induce cytotoxicity and hyperplasia but rather a retrograde (ascending) nephropathy, a lesion that is reported not to be associated with an increased risk of renal cancer in rodents. This lesion of retrograde nephropathy has been recently reported in the literature by Dr. Hard (2009) and therefore was not recognized in the original study report. RGN should be distinguished from chronic progressive nephropathy, which is a common background lesion in mouse kidneys. CPN incidence or severity was not exacerbated by the administration of pyroxasulfone (Table 4). On the other hand RGN was increased beyond background in incidence and severity as Table 5 indicates. Minimal to moderate tubule precipitate was observed in the upper portion of the inner medulla in 14/50 male mice in the high dose group of the 18-month mouse study, and in 26/49 male mice starting at 4000 ppm. This effect was also present in the one dosed female group examined, but at much lower incidence and severity (2/49 for the 2000 ppm dose group). Precipitate was not observed at 90-days or earlier (MRID 48046904 and 47701695), but one male mouse in the 12-month interim sacrifice of the low-dose (5 ppm) group in the carcinogenicity study had material of similar staining character in 4 tubules. The reviewing pathologist examined the renal parenchyma unaffected by CPN or RGN for evidence of single cell degeneration or necrosis. No degeneration or necrosis was observed in tubules at any dose of KIH-485 in the 14-day, 90-day, and 18-month studies (including the 12-month interim sacrifices). In addition, there was no evidence of an increase in tubule cell mitotic figures indicative of cell regeneration in any of the studies. The proliferative lesions identified by the reviewing pathologist in the 18 month mouse study are reported in Table 6. Solitary adenomas were observed in several groups of male mice: one in the 5 ppm dose (animal no. 318), 3 in the dose group starting at 2000 ppm (nos. 711, 718, 750), and 2 in the dose group starting at 4500 ppm (nos. 903, 956), one of which was in the 12-month interim sacrifice group. In the female mice, there was one adenoma in the interim sacrifice group at the starting dose of 4500 ppm (no. 805). All but one of the adenomas were small and all were low-grade lesions located in the cortex, particularly near the poles of the kidney. Five of the 7 adenomas were solid, lobular lesions, while 2 were papillary cystadenomas. The largest adenoma was at the kidney pole surrounded for the most part by an RGN scar. Based on the absence of any cytotoxicity or cell regeneration of tubular epithelial cells (Aoshima 2009: MRID 48046904), and the random distribution of adenomas between the dose groups, the reviewing pathologist concluded that the

renal tumors were of spontaneous origin and pyroxasulfone is not carcinogenic for mouse kidneys. Dr. Hard found that most of the proliferative lesions reported as hyperplasias in the study report appeared to be represented by dilated proximal tubules with simple hyperplastic lining which is not a precursor of renal tubular hyperplasia.

The Pathology Working Group examined the findings of the 18-month mouse study (MRID 47701710) and the relevant shorter term studies (14-day investigative study: MRID 48046904; 90-day subchronic study: MRID 47701695) in mice along with the findings of the reviewing pathologist and confirmed the conclusions of the reviewing pathologist that pyroxasulfone is not carcinogenic for the mouse kidney. The renal tubular adenomas observed in male mice were considered by the PWG to be incidental and unrelated to the administration of pyroxasulfone. Their opinion was based on the following observations:

- Absence of any cytotoxicity (degeneration or individual cell necrosis) in studies ranging from 14 days to 18 months and doses up to 15,000 ppm.
- Absence of cell regeneration leading to precursor lesions such as atypical tubular hyperplasia at all time points and doses up to 15,000 ppm.
- Lack of exacerbation of chronic progressive nephropathy, a spontaneous disease in rodents that results in cell regeneration which can result in renal tubule tumors in chronic studies.
- Lack of a clear dose response in the distribution of tumors between test substance treated groups

The CARC's consulting pathologist Dr. John Pletcher reviewed the PWG report and the reviewing pathologist's report and concurred with their findings (TXR No. 0055884).

Table 4. Incidence and severity of chronic progressive nephropathy (CPN) in an 18-month carcinogenicity study of KIH-485 in CD-1 mice (Hard 2010: MRID 48354403).

Starting dose ppm	Mice assessed*	CPN incidence and severity grade					Mean CPN grade
		0	1	2	3	4	
Males							
0	43/50	11	32	0	0	0	0.7
5	46/49	11	35	0	0	0	0.8
150	41/47	10	31	0	0	0	0.8
2000	44/50	6	38	0	0	0	0.9
4000**	49/50	13	33	0	0	0	0.7
Females							
0	36/50	22	14	0	0	0	0.4
2000	43/49	35	8	0	0	0	0.2

*Some mice could not be assessed because of other spontaneous lesions (e.g. amyloidosis) or post-mortem change
** This group was terminated at 363 days

Table 5. Incidence and severity of retrograde nephropathy (RGN) in an 18-month carcinogenicity study of KIH-485 in CD-1 mice (Hard 2010: MRID 48354403).

Starting dose ppm	Mice assessed*	RGN incidence and severity grade					Mean RGN grade
		0	1	2	3	4	
Males							
0	43/50	40	3	0	0	0	0.1
5	46/49	42	4	0	0	0	0.1
150	41/47	34	7	0	0	0	0.2
2000	44/50	11	15	12	6	0	1.3
4000**	49/50	1	16	24	8	0	1.8
Females							
0	36/50	34	2	0	0	0	0.1
2000	43/49	11	26	6	0	0	0.9

*Some mice could not be assessed because of other spontaneous lesions (e.g. amyloidosis) or post-mortem change
** This group was terminated at 363 days

Table 6. Incidence of proliferative lesions in an 18-month carcinogenicity study of KIH-485 in CD-1 mice *(Hard 2010: MRID 48354403)

Lesion type	Starting dose (ppm)				
	0	5	150	2000	4000
Males					
ATH: Atypical Tubule Hyperplasia	0	0	0	2	0
Adenoma	0	1	0	3	2
Females					
ATH: Atypical Tubule Hyperplasia	0	0	0	0	0
Adenoma	0	0	0	1	0

* Includes mice from both the interim 12-month and the terminal 18- month sacrifices

C. Non-Neoplastic Histopathological Findings

Test substance-related microscopic findings were observed in the kidney and nervous tissue of male and female mice fed 2000/1000 ppm or 2000/500 ppm of the test substance, respectively, for up to 18 months. A non-adverse decrease in the incidence and severity of cystic endometrial hyperplasia (CEH) was also observed in females at this concentration.

All other microscopic observations in the main study mice were consistent with normal background lesions in mice of this age and strain.

Kidney:

Microscopic kidney effects in males included the presence of an intratubular precipitate, as well as increased tubular epithelial nephrosis, hyperplasia, and adenoma as reported by the study pathologist (Table 7). An increase in the incidence (females) and severity (males) of chronic progressive nephropathy (CPN) was observed by the study pathologist (Table 8). A minimal to mild precipitate was observed in the medullary tubules of 19/50 2000/1000 ppm male mice.

Minimal to mild renal tubular nephrosis (TN) was observed in 2/50, 2/50, 0/50, and 37/50 male mice fed a diet containing 0, 5, 150, and 2000/1000 ppm, respectively. The TN lesion was similar to that observed in mice from the 1-year interim sacrifice. The degenerative changes included nuclear enlargement, vacuolar degeneration/necrosis, and attenuation (thinning) of the epithelial lining. Although minimal TN was observed in 7/10 of the interim females, it was not observed in any of the main study females.

Renal tubular hyperplasia were reported by the study pathologist in 1/50, 1/50, 0/50, and 8/50 males fed a diet containing 0, 5, 150, and 2000/1000 ppm, respectively, and 0/50, 0/50, 1/50, and 2/50 females fed a diet containing 0, 5, 150, and 2000/500 ppm, respectively. All cases were graded as minimal except for 1 of the 7 high-dose male cases, which was graded as mild. The incidences in males was interpreted to be test substance related at 2000/1000 ppm, while the incidences in females were all considered to be within normal limits at all dietary exposure levels.

A revaluation of the kidney pathology by an expert pathologist (Hard 2010: MRID48354403) and by a PWG (Hardisty 2011: MIRD 48354405) determined that pyroxasulfone did not induce cytotoxicity and hyperplasia but rather a retrograde (ascending) nephropathy (RGN) presumably arising secondarily from an effect in the lower urinary tract, possibly the formation of urinary solids., a lesion that is reported not to be associated with an increased risk of renal cancer in rodents. This lesion of retrograde nephropathy has been recently reported in the literature by Dr. Hard (2009) and therefore was not recognized in the original study report. RGN should be distinguished from chronic progressive nephropathy, which is a common background lesion in mouse kidneys. CPN incidence or severity was not exacerbated by the administration of pyroxasulfone (Table 4). On the other hand RGN was increased beyond background in incidence and severity as Table 5 indicates. It was concluded by the expert pathologist and by the PWG that CPN was not treatment related, but RGN was treatment related and may have been associated with urinary solids as evidenced by the intratubular precipitate that was present in the collecting ducts in the renal medulla in some test substance-treated mice. Dr. Hard found that most of the proliferative lesions reported as hyperplasias in the study report appeared to be represented by dilated proximal tubules with simple hyperplastic lining which is not a precursor of renal tubular hyperplasia. The PWG also concluded that there was no evidence of compound-induced cytotoxicity observed in the tubules of at any dose of pyroxasulfone in the 14-day (2000 or 15,000 ppm), 90-day and 18-month studies. And there was no evidence of an increase in tubule cell mitotic figures indicative of cell regeneration in any of the studies.

Table 7. Incidences of Test Substance-Related Microscopic Findings in the Kidneys of Male and Female Mice at 18 months (Study Pathologist)								
Sex:	Male				Female			
Concentration (ppm):	0	5	150	2000/ 1000	0	5	150	2000/500
Number (mice/group):	50	50	50	50	50	50	50	50
Kidneys								
Precipitate, intratubular	0	0	0	19	0	0	0	0
Tubular nephrosis	2	2	0	37	1	0	0	1
Hyperplasia, tubular	1	1	0	7	0	0	1	2
Chronic progressive nephropathy	34	39	28	39	23	26	20	44
Adenoma, renal tubular	0	1	0	3	0	0	0	0

Table 8. Incidence and Severity of Chronic Progressive Nephropathy in the Kidneys of Male and Female Mice at 18 months (Study Pathologist)								
Sex:	Male				Female			
Concentration (ppm):	0	5	150	2000/1000	0	5	150	2000/500
Number (mice/group):	50	50	50	50	50	50	50	50
Kidneys [a]	(50)1	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Chronic progressive nephropathy	34	39	28	39	23	26	20	44
Minimal[b]	25	31	27	7	16	16	11	23
Mild	5	4	0	21	3	2	1	13
Moderate	2	2	1	7	2	2	5	8
Severe	2	2	0	4	2	6	3	0

[a] (number of organs examined) in parentheses.
[b] 4-point severity scale: 1 = minimal; 2= mild; 3 = moderate; 4 = severe.

IV. TOXICOLOGY

1. Mutagenicity

Pyroxasulfone (as KIH-485) was evaluated for mutagenicity in the standard test battery, which included a bacterial assay for gene mutations, a gene mutation assay in mammalian cells, a test for clastogenicity in mammalian cells and an *in vivo* mouse bone marrow micronucleus. In addition, seven metabolites were assessed for the potential to induce

reverse gene mutations in *Salmonella typhimurium* and *Escherichia coli.* Results show that the parent compound was not mutagenic in bacteria or cultured mammalian cells. There was also no indication that the test material induced chromosome aberrations either *in vitro* or *in vivo*. Similarly, the seven metabolites were not mutagenic in bacteria. All studies were well conducted and acceptable and satisfy the OPPTS and OECD guidelines for mutagenicity testing. Based on these considerations, there is no concern for mutagenicity at this time. Summaries of the 11 assays were presented in the first CARC report on pyroxasulfone (TXR 0055356).

Two in vivo comet assays were subsequently received from the registrant. Pyroxasulfone was positive in the *in vivo* comet assay assessing its DNA reactivity in male rats at dose levels ≥1000 mg/kg (urinary bladder) and 2000 mg/kg (liver). It was also positive in the *in vivo* comet assay assessing its DNA reactivity in male mice at dose levels ≥1000 mg/kg (kidney), but not in the liver. It was concluded that while pyroxasulfone induces DNA damage in the rat liver and urinary bladder and mouse kidney, evidence of DNA damage alone is not sufficient to declare this test material as a mutagen. Additionally, DNA damage was accompanied by apoptosis and increased mitotic figures which suggest that cytotoxicity was the underlying mechanism (apoptosis eliminating DNA damaged cells and increased mitotic figures indicating cell replacement). Summaries of the rat and mouse *in vivo* comet assays are presented below:

IN VIVO COMET TEST

The DNA reactivity of pyroxasulfone (KIH-485) TGAI was assessed in an *in vivo* comet test (MRID 48033306) in male rats at dose levels of 500, 1000 and 2000 mg/kg/day. A statistically significant increase in the tail intensity was observed in the liver at 2000 mg/kg/day ($p<0.001$), compared to vehicle control values, however no statistically significant increases were observed in males treated with pyroxasulfone at 500 and 1000 mg/kg/day. Statistically significant increases in the tail intensity were observed in the urinary bladder at 1000 and 2000 mg/kg/day ($p<0.001$), however no statistically significant increase was observed in males treated with pyroxasulfone at 500 mg/kg/day. No toxicity was observed at the limit dose of 2000 mg/kg.

The DNA reactivity of pyroxasulfone (KIH-485) TGAI was assessed in an *in vivo* comet test (MRID 48033307) in male mice at dose levels of 500, 1000 and 2000 mg/kg/day. A statistically significant increase in the tail intensity was observed in the liver of mice treated with pyroxasulfone at ≥500 mg/kg/day ($p<0.01$), however as group mean values are within the current historical control range, the result was not considered to be biologically significant. Statistically significant increases in the tail intensity were observed in the kidney of mice treated with pyroxasulfone at 1000 ($p<0.05$) and 2000 mg/kg/day ($p<0.001$), however no statistically significant increase was observed in animals treated with pyroxasulfone at 500 mg/kg/day. No toxicity was observed at the limit dose of 2000 mg/kg.

2. Subchronic and Chronic Toxicity

Refer to the Pyroxasulfone CARC Report dated May 11, 2010 (TXR 0055356). The following are new studies.

14-day oral (rat)

Reference: Tsuboi, M. (2011). Electron Microscopic Examination of Rat Urinary Bladder Treated with Pyroxasulfone (KIH-485) for 14 Days [Non-GLP]. Biosafety Research Center, Foods, Drugs and Pesticides, Lab Study No.: C014 (001-567). Original report: February 19, 2010. MRID 48046905., Revised report (amendment #1) January 5, 2011, MRID 48354402

Male Crl:CD(SD) rats (5/group) were exposed to 0, 500, 2000 or 20000 ppm (0, 44, 177 and 1765 mg/kg/day) of KIH-485 by dietary route for 14 days (MRID 48354402 & 48046905). There were no deaths, abnormalities, body weight changes, food consumption abnormalities, or urinary conditions in any of the groups. SEM examination of the urinary bladder revealed no microcrystals. Small round cells in the superficial cell layer of the urothelium were increased in the 2,000 or more ppm groups. The bladders of the animals in 20, 000 ppm treatment group had numerous foci of small round cells and thickened, folded epithelium. The surfaces of the bladder epithelium were extensively lumpy when compared to those in the other treated groups. Additionally, the severity tended to rise in a dose-dependent manner. These results would indicate the hyperplastic and cytotoxic effect of KIH-485 on the bladder transitional epithelium. In conclusion, doses of 2,000 and 20,000 ppm of KIH-485 (pyroxasulfone) induced morphological changes (suggesting hyperplastic and toxic effects) in the surface of the bladder epithelium.

7-day oral (rat)

Reference: Cohen SM and Arnold, LL. 2010. The Effects of Dietary Administration of Pyroxasulfone on the Urinary Bladder of Male Rats. University of Nebraska Medical Center, Department of Pathology and Microbiology, 983135 Nebraska Medical Center, Omaha, Nebraska 68198-3135. Study No. 310, December 21, 2010. MRID 48354404.

The cytotoxic effects of short duration exposures to pyroxasulfone on the urothelium was evaluated in male Crl:CD(SD) rats (18 animals per group) exposed to 0, 50, 1000 or 2000 ppm of KIH-485 (pyroxasulfone 99.21% a.i.) by dietary route for 7 days. Six animals per group were sacrificed on days 1, 3, or 7 and the urinary bladders were removed for SEM and histochemical evaluation. One animal in the 50 ppm pyroxasulfone group sacrificed on Day 7 was diagnosed with papillary and nodular hyperplasia in the urinary bladder. There were no pyroxasulfone-induced increases in the BrdU labeling index at any concentration of pyroxasulfone on study days 1 and 3. However, on study day 7, there was a significant increase in the BrdU labeling index in the 2000 ppm group (0.25 ± 0.06%) compared to the control group (0.09 ± 0.02%).

Histopathological examination of urinary bladder showed no statistically significant incidence of changes in the bladder epithelium of rats treated with 50, 1000 or 2000 ppm pyroxasulfone However, there was morphological evidence of cytotoxic and proliferative changes in the bladder epithelium of several individual rats. As early as study day 3, there was one instance of mild focal simple hyperplasia present in the 2000 ppm pyroxasulfone group. On study day 7, there was one instance of papillary/nodular hyperplasia with diffuse moderate to severe simple hyperplasia and chronic inflammation in the 50 ppm pyroxasulfone group, and one instance of mild simple hyperplasia in the 1000 ppm pyroxasulfone group.

SEM examination revealed no differences between treatment groups sacrificed 1 or 3 days after treatment except for a single bladder from each treatment group (50, 1000, and 2000 ppm) had extensive necrosis and exfoliation. A single calcium-containing crystal was present on the surface of one control bladder and one bladder in the 50 ppm pyroxasulfone group. Multiple calcium containing crystals were present on the surface of one bladder in the 50 ppm group and one bladder in the 1000 ppm pyroxasulfone group. On study day 7, the SEM classification for the 50 ppm pyroxasulfone group was significantly different compared to the control. The surface of the bladder with a histopathological diagnosis of capillary/nodular hyperplasia had an extensive network of raised ridges and protrusions of round and polygonal cells of varying degrees of differentiation, and a calcium-containing crystal (greatest dimension 50 μm) was present on the surface of the bladder. Round carbon and oxygen-containing crystals were present on the surface of one bladder each in the 50 and 2000 ppm groups on day 1, on one bladder in the 1000 ppm pyroxasulfone group and on two bladders in the 2000 ppm pyroxasulfone group on day 3, and on one bladder each in the 50 and 1000 ppm groups on day 7. Similar crystals were found on the surface of one control bladder on day 3 only. Superficial cells with craters were observed in one Class 3 control bladder on study day 1, in one Class 3 bladder in the 1000 ppm pyroxasulfone group on study day 3 and in one Class 1 bladder in the 2000 ppm pyroxasulfone group on study day 7. However, the superficial cells with craters were much more numerous in the two pyroxasulfone-treated bladders compared to the control bladder.

3. Mode of Action Analysis for Urinary Bladder Tumors

Introduction

Carcinogenicity studies have been conducted with pyroxasulfone in Crl:CD1(ICR) mice and Crl:CD®(SD)IGS BR rats. Urinary bladder transitional cell papillomas were reported in male rats only. Male rats had statistically significant trends for urinary bladder transitional cell papillomas and combined papillomas and carcinomas, at $p < 0.01$. The occurrence of the papillomas was 1, 0, 0, 4 and 5 in the controls, 5 ppm, 50 ppm, 1000 ppm and 2000 ppm treatments, respectively. The study pathologist had identified one urinary bladder transitional cell carcinoma in one male rat administered 1000 pm of pyroxasulfone. An independent expert pathologist confirmed the findings of the study pathologist except for the carcinoma which was re-diagnosed as an adenoma (Cohen 2011: MRID 48354406).

New information has been submitted by the registrant (listed earlier) to support a mode of action for the urinary bladder tumors in male rats with a request to review these data and to reconsider the cancer classification of pyroxasulfone.

URINARY BLADDER CARCINOGENESIS

The key events in the mode of action of urinary bladder carcinogenesis are presented in Table 9. The postulated Mode of Action (MOA) is a non-genotoxic process producing increased cell proliferation resulting from site-specific cytotoxicity and the associated compensatory regenerative response leading to hyperplasia and subsequent tumors in the urinary bladder. The cytotoxicity of pyroxasulfone in the urinary tract has been demonstrated in short term exposure studies (MRID 48354402 and 48354404). Evidence of toxicity has also been evident in longer term exposure studies by the presence of red staining of the bedding (hematuria) as well as the presence of a chronic inflammatory infiltrate in the rat bladder. Cytotoxicity is likely due to excretion of high concentrations of the chemical (metabolites) in the urine leading to formation of urinary solids (crystals, calculi). A proliferative effect has been demonstrated at several time points.

Table 9. Key Events in the Mode of Action of Urinary Bladder Carcinogenesis in Male Rats

		Rat					
Key event	Dose (ppm)	1-3 days	7 days	14 days	90 days	12 -17m	20-23 m
Crystals, Craters (Associative, not key, event)	50	crystals	crystals				
	1000	craters	crystals				
	2000	crystals	crystal and craters	craters			
Calculi	1000					X	
	2000					X	X
Cytotoxicity (inflammation, morphologic changes	5						
	50	X	X				
	1000	X	X			X	X
	2000	X	X			X	X
Cell proliferation: hyperplasia BrdU labeling		X (2000 ppm)	X (≥50 ppm) X (2000 ppm)	X (2000 ppm) X (2000 ppm)	X (2500 ppm)	X (≥1000 ppm)	X (≥1000 ppm)
Tumors	1000					X	
	2000					X	X

Associative event: Formation of Urinary Bladder Crystals

The specific method by which administration of pyroxasulfone produces the cytotoxic effects on the bladder has not been conclusively identified, but considerable evidence suggests that it is due to the formation of urinary crystals and calculi. This is evident in the description of the histopathology from the various intermediate and long term studies, which describe the presence of urinary solids (calculi) in the urine of the rats administered pyroxasulfone at high doses as

well as intermittent hematuria observed by clinical examination during the course of the study. In the two year study with pyroxasulfone, a close correlation between rats with hematuria and those with calculi at the time of sacrifice was not evident, since both effects are intermittent, and the time of clinical observations and time of sacrifice are not the same. Crystal formation in the form of precipitate in the kidney tubules was also described in the 18-month mouse study (MRID 47701710). The precipitate present in the mouse kidney tubules, predominantly in the Loop of Henle, as noted by Hard, 2010 (MRID 48354403) is likely an indication of the poor solubility of this substance (or its metabolites) in the urine. According to Cohen *et al* 2011, (MRID 48354401) precipitation of a chemical can differ between species, not only quantitatively but qualitatively, as well as between sexes. Thus, it may be that in the mouse, the precipitate forms in the kidney tubules in addition to solids in the urine leading to retrograde nephropathy. Furthermore, a few mice in the 18-month bioassay had calculi in the urinary bladder, including in the controls. In the rat, it is more likely that precipitation is occurring in the urine itself, having been retained in solution until reaching the lower urinary tract. Formation of solids is much more likely in male rats than in female rats, predominantly because of the much higher concentrations of protein in male rats, largely due to the presence of large amounts of α_{2u}-globulin (Rodent Bladder Carcinogenesis Working Group, 1995; IARC Working Group, 1999 as cited in MRID 48354401). In the rats with urinary bladder papillomas, either calculi, calcium-containing crystals and/or eosinophilic amorphous material were present in the histopathologic slides of most of these bladders. Urine was not specifically examined (MRID 48354401).

In the one-week study of pyroxasulfone in male rats (MRID 48354404), SEM examination of the urinary bladders revealed the presence of urinary crystals on the surface of the urothelium of the bladders of some of the treated animals. Preliminary x-ray reflective spectroscopic analysis of these crystals demonstrated that most were not composed of the usual components in the urine. There was no evidence of calcium, phosphate or magnesium in most of these crystals, although a few calcium-containing crystals were present. It was theorized that most of the crystals are likely composed of organic material, probably as the parent compound itself, or one or more of its metabolites.

Crystals have not been identified in other studies with pyroxasulfone in rats (MRID 47701693; 47701708; 47701709; 48046903; 48046905). The reason, most likely, is that they are present only transiently within the animal's bladder itself, and/or transiently in the bladders or urine after the specimen has been collected. Cohen *et al* (MRID 48354401) stated that many crystals that occur in the urine are actually only partially insoluble, and with time they become completely solubilized as seen in the example of uracil (Shirai *et al.*, 1989 as cited in MRID 48354401). Because of this, special techniques are required to adequately evaluate the presence or absence of urinary solids in these animals (Cohen *et al.*, 2007 as cited in MRID 48354401). The poor solubility of pyroxasulfone and its metabolites, and the fact that it is mostly excreted in the urine, and thus would be concentrated in the urine, suggests that urinary solids are the most likely possibility for the induction of the cytotoxicity and necrosis. In the 1-week and 2-week studies, there was no evidence of an increase in the induction of apoptosis (MRID 48354404).

In the one-week study (MRID 48354404), there was evidence that these crystals might form at

the dose of 50 ppm, whereas in the 2-week study, there was no evidence of crystals at any dose and no effect on the urothelium at 500 ppm and below (MRID 48046903; MRID 48046905). Furthermore, there has been no evidence of an effect at the low dose of 50 ppm in any other experiment at any length of time (MRID 47701708; MRID 47701709). It is very likely the formation of the crystals at an early time point at the 50 ppm dose is only transient in nature, possibly due to a number of variables. It is postulated (MRID 48354401) that although the dietary concentrations are the same during the course of the experiment, the urinary concentration will progressively decrease over time since the consumption of the chemical actually decreases over time on a mg/kg basis. Furthermore, the animals have a tendency to drink less water during the first few days of chemical exposure which would lead to slightly more concentrated urine than would occur later in the experiment. No urinary bladder effects were observed in the 2-year bioassay at 50 ppm.

Frequent red stained cage boards and red discharge from penis (hematuria) has been reported from the one and two year chronic studies with pyroxasulfone (Table 10). This is likely due to the irritation caused by crystal formations in the urinary bladder as a result of the pyroxasulfone treatment and its elimination primarily through the urine. Pyroxasulfone is rapidly absorbed, metabolized and excreted, with urine being the major route of excretion and does not accumulate with prolonged dosage. Metabolites would be expected to be concentrated in the urine compared to blood and tissues as absorption, distribution, metabolism and excretion studies have demonstrated. Maximal concentrations in the plasma occur within 1 to 3 hours. It is immediately excreted in the urine with maximal concentrations in the urine at in the first urine collection at 6 hours (75% of all radioactivity excreted). Tissue distribution analysis shows that the liver, kidney and bladder contain the preponderance of the tissue radioactivity. The concentration in the bladder is the highest in the first few hours.

Table 10. Incidence of Urinary Bladder Irritation Findings in Pyroxasulfone Treated Male Rats[1]

52 weeks					
Dose (ppm)	0	5	50	1000	2000
Number of rats	20	20	20	20	20
Red stained cage boards	0	1	0	1	6
Discharge penis, red (hematuria)	2	0	0	1	4
104 weeks					
Dose (ppm)	0	5	50	1000	2000
Number of rats	70	69	70	70	70
Red stained cage boards	9	10	8	14	35
Discharge penis, red	3	5	1	6	19

[1]Reproduced from MRID 47948801

Key event: Formation of Urinary Bladder Calculi

In the chronic/carcinogenicity rat pyroxasulfone study (MRID 47701709), the incidence of urinary bladder calculi was rare but present. In the 1000 ppm group one male rat that died on Day 469 had severe calculus/calculi present. This animal was diagnosed by the study pathologist with a transitional cell carcinoma, but upon reexamination by an independent consultant

pathologist it was diagnosed as transitional cell papilloma. In the 2000 ppm group, two male rats had urinary calculus/calculi. This was accompanied by severe urinary mucosal hyperplasia and urinary inflammation mucosal fluid. Both animals died during the study, one on Day 498 and the other on Day 536. These calculi were not collected for analysis of the test material or its metabolites. In the one year rat study, two urinary bladder tumors occurred. A malignant transitional cell carcinoma was observed in a 1000 ppm male and was associated with kidney and bladder calculi, severe pyelonephritis with renal mucosal hyperplasia and moderate cystitis with severe mucosal hyperplasia. It was considered to be secondary to the bladder calculi and chronic irritation. A benign transitional cell papilloma was present in one 1000 ppm female. Since it was not associated with other lesions, it was considered incidental.

Key event: irritation and inflammatory lesions in the urinary bladder

There was evidence in the longer term studies (greater than 6 months) that cytotoxicity was occurring as seen by the presence of a chronic inflammatory infiltrate predominantly in the submucosa (MRID 47701708; MRID 47701709). However, no direct evidence of cytotoxicity of the urothelium could be identified by light microscopic examination in these longer term studies. In the short term studies (two weeks), examination of the urothelium by light microscopy or by scanning electron microscopy (SEM) showed an increased proliferative response, but the only direct evidence of urothelial cytotoxicity was the presence of crater-like formation on the luminal surface of the urothelium, (MRID 48046903 and 48354402). Urothelial necrosis was not observed in these studies. Cytotoxicity is frequently not detectable by light microscopy because of the involvement only of the superficial layer (MRID 48354401). For that reason, SEM is a much more sensitive technique to detect the cytotoxicity. A complicating factor in identifying cytotoxicity of the urothelium is that it can be transient in nature, with a proliferative response obscuring any evidence of the toxicity (MRID 48354401). Furthermore, the cytotoxicity can be focal and not detected in the limited sample available by light microscopic examination.

The 2-week exposure study was considered too long to identify the cytotoxic effects of pyroxasulfone on the urothelium (MRID 48354401). Consequently a study was performed examining the urothelium of male rats administered 0, 50, 1000, and 2000 ppm, with examination of the bladders after 1, 3, and 7 days (MRID 48354404). Cytotoxicity of the urothelium was manifested by SEM examination. Light microscopy and SEM showed full thickness damage of the urothelium, with microscopic ulceration, acute hemorrhage and fibrinous exudate. By day 7, there was an indication of an increase in proliferation, especially in the rats that had significant toxicity with consequent regenerative proliferation, including papillomatosis of the urothelium and acute and chronic inflammation involving the urothelium and the underlying submucosa and muscle layer. These are the typical changes that are seen with full thickness alteration of the urothelium (Fukushima *et al.*, 1981 as cited in MRID 48354401). If there is only toxicity and death involving the superficial layer, an inflammatory response does not occur. The basement membrane of the urothelium must be breached for an inflammatory reaction to occur. The lower incidences of chronic inflammation in the two year study compared to the incidence of hyperplasia suggests that at least some of the rats had cytotoxicity that was only superficial and was not sufficient to produce an inflammatory reaction (MRID 48354401).

Based on these observations, the mode of action for the bladder effects of pyroxasulfone in male rats is induction of cytotoxicity with necrosis and consequent regenerative proliferation. These changes appear to be focal and transient.

Key event: Epithelial Hyperplasia in the Urinary Bladder (mucosal epithelial hyperplasia, urothelial hyperplasia)

Epithelial Hyperplasia of the urinary bladder of rats was seen in several studies following administration of pyroxasulfone for varying durations. Thus, diffuse mucosal hyperplasia of the urinary bladder was observed in all male rats and in 20% of female rats administered 2500 ppm in their diet for 90 days while was none was observed at lower doses of 0, 25 or 250 pm (MRID 47701693) (Table 11).

Table 11. Incidences of Test Substance-Related Microscopic Findings in the Urinary Bladder in Male Rats Administered Pyroxasulfone for 90 days [a]

	Male				Female			
Concentration (ppm)	**0**	**25**	**250**	**2500**	**0**	**25**	**250**	**2500**
No. Rats	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Urinary Bladder								
Hyperplasia, mucosal, diffuse	0	0	0	10	0	0	0	2

[a] Data were obtained from text table on page 33 and Table 40 on pages 147 and 158 of MRID 47701693.

In the one year rat study (MRID 47701708), minimal to severe focal and diffuse mucosal hyperplasia of the urinary bladder was observed in 1000 (17/20) and 2000 (18/20) ppm male and 2000 (11/20) ppm female rats (Table 12). In the controls, there were two cases of mucosal hyperplasia in males and one case in females. The urinary bladder mucosal hyperplasia was characterized by a focal, multifocal or diffuse increase in the thickness of the transitional epithelium (urothelium) lining the urinary bladder lumen. The hyperplasia was identical to that observed in rats secondary to either urinary tract infection-associated inflammation or bladder calculi-associated irritation. In addition, hyperplasia of the urinary bladder may occur in control animals in the absence of either inflammation and/or calculi. Red stained urine was observed in the high dose males. In both sexes urinary bladder mucosal inflammation was occasionally observed in rats with mucosal hyperplasia).

Table 12. Incidences of Test Substance-Related Non-neoplastic Findings in the Urinary Bladder Male and Female Rats Administered Pyroxasulfone for One Year					
Concentration (ppm):	0	5	50	1000	2000
Number of Rats/Sex:	**20**	**20**	**20**	**20**	**20**
Male					
Urinary Bladder	(20)	(20)	(20)	(20)	(20)
Hyperplasia, mucosal, diffuse (Total)	2	0	0	14**	16**
Minimal	1	0	0	5	1
Mild	1	0	0	5	14
Moderate	0	0	0	3	1
Severe	0	0	0	1	0
Hyperplasia, mucosal, focal/multifocal (Total; Minimal)	0	0	0	3	2
Inflammation, mucosal (Total)	1	0	0	3	3
Minimal	1	0	0	2	3
Moderate	0	0	0	1	0
Female					
Urinary Bladder	(20)	(20)	(19)	(19)	(20)
Hyperplasia, mucosal, diffuse (Total)	1	2	2	0	4
Minimal	0	1	2	0	2
Mild	1	0	0	0	0
Moderate	0	0	0	0	1
Severe	0	1	0	0	1
Hyperplasia, mucosal, focal/multifocal (Total; Minimal)	0	0	0	0	7**
Inflammation, mucosal (Total)	1	1	1	0	2
Minimal	0	0	1	0	1
Mild	1	0	0	0	0
Moderate	0	1	0	0	0
Severe	0	0	0	0	1

Data were obtained from pages 136-150 and 184-206 of Final Report, DuPont 19621, Vol-1. MRID 47701708

In the two year study, in males, increased ($p<0.01$) incidences of minimal to severe mucosal hyperplasia were noted in the 1000 ppm (31%) and 2000 ppm (61%) groups compared to the controls (6%), and an increased ($p<0.01$) incidence of minimal to mild mucosal inflammation was observed at 2000 ppm (14%) compared to the controls (4%) (Table 13). In females, the incidence of these lesions was low and comparable among all groups (MRID 47701709).

Table 13. Incidences of Test Substance-Related Non-neoplastic Findings in Male and Female Rats Administered Pyroxasulfone for Two Years (MRID 47701709)

Concentration (ppm):	0	5	50	1000	2000
Number of Rats/Sex:	70	70	70	70	70
Male					
Heart	(70)[1]	(70)	(70)	(70)	(70)
Cardiomyopathy (Total)	50	47	53	60	62
Minimal	22	15	19	18	10
Mild	22	21	24	30	24
Moderate	5	11	9	12	23
Severe	1	0	1	0	5
Urinary Bladder	(70)	(69)	(70)	(70)	(70)
Hyperplasia, mucosal (Total)	4	5	2	22**	43**
Minimal	2	3	1	9	15
Mild	1	2	0	9	19
Moderate	0	0	1	3	7
Severe	1	0	0	1	2
Inflammation, mucosal (Total)	3	2	0	6	10**
Minimal	2	1	0	3	3
Mild	1	1	0	1	7
Moderate	0	0	0	2	0
Female					
Heart	(70)	(70)	(70)	(70)	(70)
Cardiomyopathy (Total)	32	23	32	43	48
Minimal	26	19	19	21	18
Mild	6	3	11	17	17
Moderate	0	1	2	4	11
Severe	0	0	0	1	2
Sciatic Nerve	(69)	(70)	(69)	(70)	(70)
Degeneration, axon/myelin (Total)	8	6	10	15*	20**
Minimal	8	6	9	14	19
Mild	0	0	1	1	1
Liver	(70)	(70)	(70)	(70)	(70)
Peribiliary fibrosis/inflammation (Total)	14	11	21	23*	29**
Minimal	10	9	17	19	22
Mild	4	2	4	4	7
Hyperplasia, bile duct (Total)	14	10	13	14	23
Minimal	11	8	7	11	16
Mild	3	2	6	3	7

[1] (number of organs examined) in parentheses.
* Significantly different ($p<0.05$) from the control groups
** Significantly different ($p<0.01$) from the control groups

In a multigeneration study with rats (MRID 47701706), test substance-related microscopic findings were observed in the urinary bladder (diffuse mucosal hyperplasia) of the 2000 ppm P and F1 adult males and females (Tables 14 and 15). In males only, urinary bladder hyperplasia was usually associated with minimal mucosal inflammation. The following treatment-related findings were increased in incidence compared to 0 controls (i) mucosal hyperplasia in the P males (25/30) and females (16/30) and F1 males (24/26) and females (19/28); and (ii) minimal mucosal inflammation in the P males (25/30, 1 severe) and F1 males (10/26).

Table 14. Incidences of Test Substance-Related Microscopic Findings in P Male and Female Adult Rats (# animals affected)

	Male				Female			
Concentration (ppm)	**0**	**5**	**100**	**2000**	**0**	**5**	**100**	**2000**
Number of rats	**30**	**29**	**30**	**30**	**30**	**30**	**30**	**30**
Liver	(30)	(29)	(30)	(30)	(30)	(30)	(30)	(30)
Hypertrophy, hepatocellular	0	0	0	26	0	0	0	16
Minimal	0	0	0	15	0	0	0	14
Mild	0	0	0	11	0	0	0	2
Heart	(30)	(28)	(30)	(30)	(30)	(29)	(30)	(29)
Cardiomyopathy	7	7	8	11	0	0	3	14
Minimal	5	6	7	8	0	0	2	7
Mild	2	1	1	2	0	0	1	6
Moderate	0	0	0	1	0	0	0	1
Urinary Bladder	(29	(29)	(30)	(30)	(30)	(30)	(30)	(30)
Hyperplasia, mucosal, diffuse	0	1	0	25	0	0	0	16
Minimal	0	1	0	7	0	0	0	14
Mild	0	0	0	16	0	0	0	2
Moderate	0	0	0	1	0	0	0	0
Severe	0	0	0	1	0	0	0	0
Inflammation, mucosal	0	1	0	25	0	0	0	1
Minimal	0	1	0	24	0	0	0	1
Severe	0	0	0	1	0	0	0	0

Data were obtained from Tables 83 and 84 on pages 181-186. The number of tissues examined is included in parentheses, MRID 47701706.

Table 15. .Incidences of Test Substance-related Microscopic Findings in F1 Male and Female Adult Rats (# animals affected)

	Male				Female			
Concentration (ppm)	**0**	**5**	**100**	**2000**	**0**	**5**	**100**	**2000**
Number of rats	**30**	**29**	**30**	**30**	**30**	**30**	**30**	**30**
Liver	(30)	(29)	(30)	(28)	(30)	(30)	(29)	(29)
Hypertrophy, hepatocellular	0	0	0	24	0	0	0	16
Minimal	0	0	0	17	0	0	0	16
Mild	0	0	0	7	0	0	0	0
Heart	(30)	(30)	(30)	(28)	(30)	(30)	(30)	(29)
Cardiomyopathy	5	7	8	9	2	0	0	18
Minimal	5	6	4	2	2	0	0	5
Mild	0	1	4	5	0	0	0	6
Moderate	0	0	0	2	0	0	0	3
Severe	0	0	0	0	0	0	0	4
Urinary Bladder	(27)	(26)	(29)	(26)	(30)	(30)	(30)	(28)
Hyperplasia, mucosal, diffuse	0	1	0	24	0	0	0	19
Minimal	0	1	0	9	0	0	0	13
Mild	0	0	0	6	0	0	0	6
Moderate	0	0	0	9	0	0	0	0
Inflammation, mucosal	0	0	0	10	0	0	0	1
Minimal	0	0	0	10	0	0	0	1

Data were obtained from Tables 85 and 86 on pages 188-192. The number of tissues examined is included in parentheses, MRID 47701706

Key Event: Neoplastic Lesions in the Urinary Bladder

In rats administered pyroxasulfone in the diet for up to 23 months, male rats developed transitional cell papilloma at the 1000 ppm (4/68) and 2000 ppm (5/66) compared to 1 papilloma in the control and non at the lower doses of 5 or 50 ppm.

Summary and Time Line of Key Events in the Proposed Mode of Action

The temporal sequence of key events in the mode of action for induction of urinary bladder tumors in pyroxasulfone-treated animals is presented in Table 16.

TABLE 16. Temporal Sequence of Key Events in Animals Administered Pyroxasulfone

Species	Event
	1 Week
Rat	Urinary crystals on the surface of the urothelium of the bladder (males) identified using SEM. Urinary crystals were not seen in other studies probably being transient. The poor solubility of pyroxasulfone and its metabolites, mostly excreted in the urine, suggests that urinary solids are the most likely possibility for the induction of the cytotoxicity and necrosis. Calculi detected after 12 months strongly suggest the formation of crystals.
	2 Weeks
Rat	urothelial cytotoxicity evident by the presence of crater-like (erosion) formation on the luminal surface of the urothelium
	90 Days (13 Weeks)
Rat	Mucosal hyperplasia of the urinary bladder in all male rats at the high dose (2500 ppm)
	180 Days
Rat	Diffuse mucosal hyperplasia of the urinary bladder >80% of the P and F1 males in the 2000 ppm and >50% in the 2000 ppm P and F1 females. Urinary bladder mucosal inflammation.
	12 Months
Rat[a]	Focal and diffuse mucosal hyperplasia of the urinary bladder >80% of the males in the 1000 and 2000 ppm and 50% in the 2000 ppm females. Urinary bladder mucosal inflammation. Mucosal epithelial hyperplasia (males & females)
	13-23 Months
Rat	**Calculus/Calculi** (males) Mucosal epithelial hyperplasia (males) Mucosal inflammation Transitional papilloma (males)

Human relevance of transitional cell papillomas in the urinary bladder

Urothelial toxicity caused by crystal and calculus/calculi leading to urinary bladder tumors is possible in humans. There is considerable evidence in the literature that crystal formation occurs more readily in rats than in mice, and in male rats more likely than in female rats (IARC Working Group, 1999 cited in MRID 48354401). This was the case for pyroxasulfone.

However, for a given chemical, this is dependent on the variables that influence its precipitation in the urine, including the toxicokinetics of the chemical and its metabolites as well as the species.

Alternative MOAs

The induction of bladder tumors by increasing cell proliferation can occur either by an increase in cell death or by direct mitogenic activity. The evidence presented supports the cytotoxicity mechanism with consequent regeneration rather than the mitogenic effect. This has been demonstrated in the one week study (MRID 48354404).

The other alternative mechanism would be through genetic changes. Pyroxasulfone was not mutagenic. Pyroxasulfone was positive in the comet assay assessing DNA reactivity in rat livers and urinary bladders and mouse kidneys at extremely high doses compared to the doses employed in the oncogenicity bioassays. It is likely, however, that the DNA damage results from cytotoxicity. In the absence of mutagenic response (i.e., gene mutation or structural chromosome aberrations) in well-done guideline studies, it is likely that this DNA damage is not repaired. Hence, the additional evidence of apoptosis (to remove the damaged cells) and mitotic figures (to replace the damaged cells) is indicative of a cytotoxic response.

Conclusions

There is strong evidence that treatment with pyroxasulfone leads potentially to crystal formation in the urinary tract. Evidence for the crystal formation comes from calculi detected in the urinary bladder, and the abrasive action of calculi in the bladder epithelium causing irritation (erosion and ulceration) and inflammatory lesions in the urinary bladder in addition to frequent presence of red urine and red discharge from the penis. Urinary crystals on the surface of the urothelium of the bladder (males) were identified using SEM in a one week study in male rats. Urinary crystals were not seen in other studies probably due to a transient nature of the crystal formations. Destruction of the mucosal epithelium leads to a regenerative (cell proliferation) response and epithelial hyperplasia. Transitional cell neoplasms are generally associated with calculi and epithelial hyperplasia in the urinary bladder. Transitional cell neoplasms were found in four and five male rats administered 1000 ppm and 2000 ppm in the diet but not in females. In addition, mucosal hyperplasia, accompanied mostly with mucosal inflammation was seen in male rats mostly as early as 90 days after exposure to pyroxasulfone. Therefore, this evidence suggests that the transitional cell neoplasms in male rats are related to treatment with pyroxasulfone.

The evidence showed that the development of urinary bladder tumors is a high-dose threshold phenomenon. Mucosal hyperplasia, mucosal inflammation and urinary bladder tumors were found only in animals administered high doses ($\geq$1000 ppm). Urinary calculi were also seen at the high doses. Therefore, the evidence supports a non-linear (threshold) mode of action with development of calculi, mucosal irritation and hyperplasia being a prerequisite for development of urinary bladder tumors in pyroxasulfone -treated males. These tumors are considered to be

relevant to humans.

V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

At the re-evaluation, the Committee considered the following for a weight-of-evidence determination on the carcinogenic potential of pyroxasulfone:

Carcinogenicity

Rats

- *Urinary Bladder Tumors:* Administration of pyroxasulfone resulted in transitional cell bladder tumors in male rats. Dr. Samuel Cohen, an international expert in the field of renal pathology, re-evaluated the histopathology findings from the chronic toxicity/carcinogenicity rat study and confirmed the tumor findings, except for one carcinoma at the 1000 ppm dose that he diagnosed to be an adenoma. Male rats had statistically significant trends for urinary bladder transitional cell papillomas and combined papillomas and carcinomas, both at $p < 0.01$. Although not significant by pair-wise comparison at the top two doses, the incidence of urinary (urothelial) papillomas in male Crl:CD (CD) rats at 1000 ppm (6%) and 2000 ppm (8%) exceeded the historical control range of 0-3.33% from the animal provider (Charles River Laboratories) and the laboratory historical control. The urinary bladder transitional cell tumors are considered to be rare tumors. Supporting evidence for these tumors included preneoplastic lesions (mucosal hyperplasia and inflammation) seen at 1000 and 2000 ppm. **The CARC considered the transitional cell bladder tumors in male rats to be treatment-related.**

Mouse

- *Kidney Tumors:* Kidney tubule adenomas occurred in 1/49 and 3/44 male mice administered 5 and 2000 ppm of pyroxasulfone in the diet. None were reported at the mid-dose of 150 ppm or the controls. Dr. Gordon Hard, an international expert in the field of mouse kidney tumors, re-evaluated the histopathology findings from the chronic mouse study along with 14- and 90-day mouse studies and confirmed the tumor findings. However, Dr Hard concluded that these tumors were spontaneous and not treatment-related. A Pathology Working Group (PWG) of five prominent pathologists including the study pathologist and Dr. Hard and chaired by Dr. Hardisty reaffirmed Dr. Hard's findings and conclusions. These conclusions were based on the following considerations: 1) Absence of any cytotoxicity (degeneration or individual cell necrosis) in studies ranging from 14 days to 18 months at doses up to 15,000 ppm; 2) Absence of cell regeneration leading to precursor lesions such as atypical tubular hyperplasia at all time points and doses up to 15,000 ppm; 3) Lack of exacerbation of chronic progressive nephropathy, a spontaneous disease in rodents that results in cell regeneration which can result in renal tubule tumors in chronic studies; and 4) Lack of a clear dose response in the distribution of tumors between test substance treated groups. **The CARC considered**

the evidence presented in this PWG re-evaluation and concurred with the PWG conclusion that the kidney adenomas in male mice were not treatment-related.

Mutagenicity

- There is no concern for mutagenicity.

Mode of Action (Male Rat Urinary Bladder Tumors)

- The postulated Mode of Action (MOA) for urinary bladder tumors in male rats is a non-genotoxic process producing increased cell proliferation resulting from site-specific cytotoxicity and the associated compensatory regenerative response leading to hyperplasia and subsequent tumors in the urinary bladder. The series of events leading to urinary bladder tumors (transitional cell papillomas) are initiated by the formation of crystals in urine and the formation of calculi which induce a hyperplastic (preneoplastic) response in the urinary bladder epithelium. Urinary bladder epithelial hyperplasia is a regenerative response resulting from irritation and inflammation caused by an abrasive action of calculi on the urinary bladder epithelium. Urinary bladder lesions that precede or accompany epithelial hyperplasia may include inflammation (acute or chronic), ulceration, and necrosis. Crystal formation in the absence of calculi is not associated with hyperplasia or urinary bladder tumors; therefore, the formation of urinary bladder calculi is the prerequisite for subsequent hyperplasia and neoplasia. The requirement for calculi formation also supports high-dose threshold phenomenon for the development of urinary bladder tumors, i.e., tumors do not develop at doses too low to produce calculi. The CARC concluded that the mode of action for bladder tumors has been adequately established. The data presented supported both a dose-response and temporal concordance of the key events and bladder tumors.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with EPA's *Final Guidelines for Carcinogen Risk Assessment (March 2005)*, the CARC classified Pyroxasulfone as "Not Likely to be Carcinogenic to Humans" at doses below those that cause urinary bladder calculi formation resulting in cellular damage of the urinary tract. Crystal formation in the absence of calculi is not associated with hyperplasia or urinary bladder tumors; therefore, the formation of urinary bladder calculi is the prerequisite for subsequent hyperplasia and neoplasia. The requirement for calculi formation also supports a high-dose threshold phenomenon for the development of urinary bladder tumors, i.e., tumors do not develop at doses too low to produce calculi. No treatment-related tumors were seen in mice. There is no concern for mutagenicity.

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The Agency has determined that the quantification of risk using a non-linear approach (i.e., RfD) will adequately account for all chronic toxicity, including carcinogenicity, that could result from exposure to pyroxasulfone. There is a clear threshold of 1000 ppm (42.55 mg/kg/day) for tumorigenesis. A point of departure (POD) of 50 ppm (2.05 mg/kg/day) is not expected to result in urinary bladder calculi formation which is a prerequisite for subsequent hyperplasia and neoplasia.

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